Chemo-Immunological Studies in Pneumococcus Infection and Immunity.

The disease acute lobar pneumonia, which ranks high among the leading causes of death in this country, is due in the great majority of instances to a microorganism commonly called the Pneumococcus. Studies of this disease carried out in wards and laboratories of the Hospital have been directed toward acquiring a better understanding of the interactions between host and parasite upon which the outcome of the infection depends. To this end our efforts have centered in the attempt to reconstruct for this particular microorganism a more precise knowledge of the biological properties peculiar to it and the nature of the protective processes which the animal body develops against it.

It now appears that specific differences in the immunological reactions of various types of Pneumococcus are related to fundamental differences in the chemical nature of the separate cell constituents. The correlation between the chemistry of the bacterial cell and its biological activities as a parasite is becoming increasingly important. Recent studies suggest "that in the past we may have regarded bacteria too much as a whole without appreciating that they consist of separate component parts of diverse function and composition." The total immune response of the host comprises not alone a reaction to the bacterial cell as a whole, but in addition the specific and individualized responses of its chemically distinct and biologically specific constituents.

To illustrate the advance in our knowledge which has grown out of studies in this newly developing field of chemo-immunology, may I recall certain facts concerning a single component of Pneumococcus and point out the intimate relation which this substance bears to the morphological structure and biological activities of the cell as a whole.

It will not be necessary here to recall in detail the earlier studies of that large group of investigators who both in this country and abroad have contributed so largely to this problem, save in so far as the facts they have revealed are related to and form the basis of the more recent work. You are already familiar with the renewed impetus that was given the study of pneumonia through the working out of the biological classification of Pneumococcus which made possible the recognition of sharply defined and specific types within this previously confused group of microorganisms. You will recall that by the application of this method it has been possible to determine the frequency of occurrence of these specific types in pneumonia and to recognize differences in the severity and mortality of the infections they produce; that a study of the presence of the classifiable types of Pneumococcus in the mouth secretions of healthy individuals has revealed a mode of dissemination of the disease-producing varieties by healthy carriers and convalescents, and that this mechanism of spread has suggested a new interpretation of the epidemiology of the disease; and finally that the knowledge of type-specificity among pneumococci has furnished a rational basis for the development of an immune serum which in the treatment of Type I infection at least has proved of distinct therapeutic value.

The biological classification was worked out before we had any knowledge of the chemical nature of the substance upon which these specific relationships are now known to depend. In the course of these studies we were naturally led to inquire as to the nature of this extraordinary specificity; upon what constituent of the cell it depends and why organisms which are morphologically so identical should differ so markedly in their immunological properties. The answer to these questions lies in the fact now recognized that the selective specificity of these bacterial types depends upon the presence in the cell of a specific substance which is distinctive for each type. This substance has been isolated and chemically identified, its origin has been traced to a definite structural part of the bacterial cell and its elaboration has been found to be a special function of organisms that are especially adapted to growth in the animal body.

Under suitable cultural conditions and particularly when they grow in the tissues of a susceptible host, pneumococci develop about them a peripheral layer of material which ensheathes their bodies in an enveloping structure known as the cell capsule. During growth these encapsulated cells release into the medium of their environment a diffusible substance which in soluble form retains the type-specificity of the organisms from which it is derived. This soluble specific substance is found not only in the cell-free filtrates of young cultures but also in the body fluids of experimental animals and in the blood and urine of patients during the course of pneumococcus pneumonia. The function of elaborating this specific material is most highly developed in the most virulent forms. There is ground for the belief that the capsules of the virulent cells are composed largely of this soluble specific substance. Thus, there is disposed peripherally about the bacteria an ectoplasmic layer of capsular material which reacts in a specific manner with the serum of an immunized animal. This reaction is remarkably specific, occurring only when the antiserum and the reacting substance are both of the same specific type. These immunological reactions form the basis of the original classifications. But the actual isolation of these specific substances, the determination of their chemical constitution, and their relationship to pneumococcus infection and immunity are more recent developments in the solution of the problem.

It may now be confidently stated that the substances which distinguish the specific types of Pneumococcus are chemically complex sugars - polysaccharides - and that they have their origin in the capsular mechanism of the cell. No matter from what type of Pneumococcus these substances are isolated, they all possess in common the chemical properties of complex sugars. But interestingly enough, the sugar derived from each specific type of Pneumococcus is chemically distinct, possessing unique properties which serve to distinguish it from the others. These sugars, when chemically isolated and purified, still retain the capacity to react specifically in antipneumococcus serum. They are chemically different and serologically specific for each type of Pneumococcus. Type-specificity, therefore, is determined by the chemical individuality of the particular polysaccharide present in the cell capsule.

The fact that immunological specificity is determined by a carbohydrate is all the more striking since previously only proteins were thought to participate in the immunity reactions.

The fact that polysaccharides, elaborated by the bacteria at the focus of disease, are found in the blood and are excreted in the urine unchanged in specificity is evidence that they are diffusible substances for which the body possesses no enzyme capable of breaking them down into simpler and inert sugars. The significance of this fact will become more evident when later on we shall discuss the protective action of a bacterial enzyme recently discovered which is capable of decomposing the capsular polysaccharide of Type III Pneumococcus in the animal body.

Although there is no direct proof that the capsular polysaccharides of Pneumococcus are primarily toxic and directly responsible for the intoxication that accompanies the disease, there is evidence that indirectly at least they may have a harmful effect on the natural processes of recovery. Because of the avidity with which they unite with antibodies, they tend to neutralize the immune substances in the blood and thus to prevent these protective agents from reaching the infected areas. Moreover, the capsular polysaccharides are known to inhibit phagocytosis, a protective process by which the white blood cells seek to destroy and remove the invading bacteria from the body. Encapsulated pneumococci surrounded by an ectoplasmic layer of carbohydrate material resist phagocytosis, while the same organisms stripped of the capsular substance and existing as naked cells are readily engulfed by the phagocytes. This fact confirms the old observation that capsule formation is a protective mechanism on the part of the bacterial cell by means of which it seeks to protect itself against the cellular defense of the host.

Fully virulent and encapsulated pneumococci, when subjected to an unfavorable environment, may lose these specific characteristics and become degraded into non-virulent forms, without capsules and without the immunological specificity that distinguished their parasitic antecedents. However, these relatively avirulent degraded forms may again revert to highly virulent encapsulated organisms. The fact that the non-invasive variants of Pneumococcus are potentially capable of regaining virulence under favorable conditions is a matter of considerable biological and epidemiological importance. It suggests the possibility that the degraded and attenuated forms may not, under all circumstances, remain the harmless saprophytes they are generally thought to be.

The facts which I have thus briefly outlined will serve to indicate the significance of specific carbohydrates in the structure and function of the bacterial cell. We have seen that the specificity of the recognized types of Pneumococcus depends on the presence of a particular carbohydrate in the cell capsule; that the elaboration of this specific sugar is a function most highly developed in form adapted to growth in the animal body; that when this function is inhibited or suppressed, the bacteria lose their capsules and revert to the common undifferentiated forms of the species; that these degraded and relatively harmless variants are capable of again developing into highly virulent encapsulated organisms; and that the acquisition of virulence and specificity is invariably associated with the regaining of the function of elaborating the capsular polysaccharide.

When the capsular polysaccharides are isolated as chemically pure substances, they still retain unimpaired the property of reacting specifically in an immune serum of the homologous type, but they lose more or less completely the property of stimulating the formation of antibodies when injected into animals. Since in the native state in which they exist as a part of the bacterial cell they function as true antigens in producing antibodies, it appears that they must exist there not as free polysaccharides but in some other form possibly combined with some substance which confers upon them the properties of a true antigen.

It has long been recognized that simple sugars such as glucose do not possess the property of an antigen, that is, they are incapable of stimulating the formation of antibodies in the animal body. However, it is now known that if these sugars are combined by chemical means with a protein, that is, with a substance naturally endowed with antigenic properties, the new sugar compounds thus formed incite the formation of antibodies that are specific for the particular sugar used. The study of synthetic antigens prepared by combining a simple sugar with protein has shown that the specificity of the newly formed compounds is determined by the chemical individuality of the reactive carbohydrate irrespective of the protein to which it is attached. Antisera produced by immunization with these conjugated sugar-proteins invariably reflect the controlling influence of the carbohydrate on the specificity of the whole compound. The studies on the simple non-bacterial sugars emphasize again the significance of carbohydrates in orienting the specific immune response of the body to substances of this class.

The results of this work led us to test the possibility of synthesizing an artificial pneumococcus antigen by combining the capsular polysaccharide with a foreign protein. For this purpose the polysaccharide of Type III was chosen since in its purified form it contains no nitrogen and represents a definite chemical entity. Further, if results were obtained with this particular sugar, they would be all the more interesting since the isolated pure substance itself has never been found to elicit antibodies in rabbits and even the intact cells from which it is derived frequently fail to incite antibody formation in these animals. By an intricate chemical synthesis, the details of which need not concern us here, it was found possible to combine the Type III capsular polysaccharide in stable chemical union with an unrelated protein of animal origin. This artificial antigen has in common with Type III Pneumococcus only the capsular polysaccharide, the protein with which it was combined being of widely remote biological origin. Rabbits injected with this artificial antigen were actively immune to subsequent infection and their serum specifically agglutinated living cultures of Type III Pneumococci, precipitated solutions of Type III polysaccharide, and protected mice against Type III Pneumococcus infection. In other words, this synthesized compound, containing only a single component of the pneumococcus cell, called forth an immune response as specific in nature as that induced by the whole microorganism.

Having found that the capsule of the virulent types of Pneumococcus consists of a polysaccharide, it occurred to us that there might exist in nature, as it were, an enzyme capable of decomposing this complex sugar. For the past several years a systematic search for an agent of this nature has been carried on. Various enzymes of animal and plant tissue and a number of different species of bacteria, molds, yeasts, and Actinomyces, many of which were known to decompose other complex carbohydrates, were tested without success. Recently, however, from soil a bacillus has been isolated which possesses an enzyme capable of decomposing the capsular polysaccharide of Type III Pneumococcus. This enzyme can be extracted in soluble form from the bacilli. When a sterile extract containing the active enzyme is added to a solution of the Type III polysaccharide, the sugar is rapidly decomposed and loses all of its specific properties. The enzyme is specific in its action, decomposing only the capsular substance of Type III; it has no effect on the capsular carbohydrates of Type I or II, which, although belonging to the same class of complex sugars, are individually different in chemical constitution.

The enzyme acts not only on the chemically isolated polysaccharide but it has the same destructive action on the capsular substance of living pneumococci when growing in the culture tube or in the tissues of infected animals. Its action in combatting infection in treated mice and rabbits is being carefully studied. In repeated experiments it has been found that a single injection of active enzyme protects mice against infection with a million times the fatal dose of a virulent culture of Type III Pneumococcus. The mechanism underlying this protective action appears to rest on the capacity of the enzyme to decompose the capsular substance surrounding the living pneumococci, thus exposing the naked bacterial bodies, stripped of their capsular defense, to the direct attack of the phagocytic cells of the host.

Recent experiments show that the specific enzyme not only protects animals if administered at the time of infection, but that it also has a curative action on an infection already well established before treatment is begun. For example: a small quantity of a virulent culture of Type III Pneumococcus injected into the skin of a rabbit produces a marked and progressive inflammatory lesion at the site of inoculation. From this local focus of infection, pneumococci rapidly invade the blood stream, setting up a septicemia which increases until death occurs, often within 72 hours. Rabbits, infected with much larger amounts of culture and treated only after the lapse of 24 hours, have recovered completely following a single adequate dose of the active enzyme.

Before the therapeutic limitations of this specific agent can be accurately defined, a much further study must be made of its use in larger animals, such as monkeys, in which it is possible to induce a disease of the lungs that in clinical course and pathology more closely resembles pneumococcus pneumonia in man.

These studies emphasize the fact that the capsule - long recognized as a defense mechanism on the part of virulent bacteria - is a decisive factor in determining the fate of pneumococci in the animal body and that this structure is vulnerable to attack by biologically specific agents.

In this brief review I have attempted to point out the significance of complex sugars in the chemical structure and biological activities of pneumococci; how the capsular polysaccharides determine the specificity and invasiveness of these organisms and influence the immunity responses of the host; and finally, the relation which these chemical and immunological facts bear to the problems of pneumococcus infection and immunity.

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